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Exhaled nitric oxide levels in school children in relation to IgE sensitisation and window pane condensation

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Summary
Background: A positive relation between exhaled nitric oxide (NO) levels and allergen exposure has been found in some studies whereas there is less information on how non-allergen environmental factors influences exhaled NO.
Objective: To study the relationship between exhaled NO levels in schoolchildren in relation to IgE sensitisation and allergenic and non-allergenic environmental factors.
Method: This study comprised 374 schoolchildren (13–14 years of age) who performed exhaled NO-measurements and skin prick tests. Exposure to allergens, respiratory infections, environmental tobacco smoke and home window pane condensation, the latter an indicator of high humidity and poor ventilation was evaluated through questionnaires.
Results: In IgE-sensitised children sensitisation to pets was a more important determinant of exhaled NO than sensitisation to pollen. Higher NO levels were found in cat-sensitised children with a cat or other furred pets at home compared to cat-sensitised children without pets (geometric mean, 24.0 vs. 13.9 ppb, $P = 0.03$). Significantly higher exhaled NO levels were found in non-sensitised children that reported having a cold (5.7 vs. 3.8 ppb, $P < 0.001$) or lived in homes with window pane condensation (7.1 vs. 4.4 ppb, $P = 0.01$) than in non-sensitised children without

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a cold and window pane condensation, respectively. These associations were not found in children that were sensitised to inhalation allergens.
Conclusion: Allergen exposure seems to be the most important determinant for exhaled NO levels in IgE-sensitised children whereas in non-sensitised children NO levels were associated with respiratory infections and home window pane condensation.

Introduction

Asthma is defined as an inflammatory disease and anti-inflammatory treatment is the corner stone of modern asthma treatment. A problem in the clinical management of asthma is that it is difficult to monitor airway inflammation in a clinical setting. One method that has been introduced for non-invasive monitoring of airway inflammation is measuring exhaled nitric oxide (NO). The level of exhaled NO is increased in asthmatics[1–3] and has been found to correlate with the level of airway inflammation[4,5] and bronchial responsiveness to agents such as methacholine[6,7] histamine[3] and hypertonic saline.[8]

In studies of subgroups of patients with asthma the exhaled NO levels are higher in patients with allergic than patients with non-allergic asthma.[7,9,10] Increased exhaled NO has also been found in patients with allergic rhinitis[10,11] and in some studies a relationship between allergen exposure and exhaled NO has been found.[11–13] Other factors that may influence the level of exhaled NO are viral respiratory infections.[14,15] There are also indications that outdoor air pollution[16] and indoor environmental factors may influence exhaled NO.[17]

The aim of this investigation was to study the relationship between exhaled NO levels in relation to IgE sensitisation, respiratory infections and allergenic and non-allergenic environmental factors in schoolchildren.

Population and methods

General design

This study was performed in schools of Uppsala community, Sweden, from October 1998 to December 1999. A short questionnaire was distributed to 13–14-year-old pupils and their parents in nine randomised schools in the community together with a letter of information. Nine-hundred and fifty-nine children (83%) and their parents agreed to participate, and were finally included.[18]

At the schools the pupils filled in the symptom part of the questionnaire, performed exhaled NO measurements and spirometry and participated in a short structured interview. In a sub-sample of the children skin prick test was performed. The sub-sample consisted of all participants from two of the schools ($n = 207$) and children from the other schools who were selected because they have symptoms suggestive of asthma or high exhaled NO-values (fraction of expired NO) at the exhalation flow rate $= 0.1$ L/s ($FE_{NO0.1}$)$\geqslant 15$ ppb) ($n = 167$). The analyses in this paper only include children where skin prick test had been performed ($n = 374$).

Questionnaires

The parents completed one questionnaire that dealt with heredity and environmental issues. In the schools the children filled in a questionnaire translated into Swedish, previously used as part of the world-wide International Study of Asthma and Allergies in Childhood (ISAAC) study.[19] The study nurse performed a short structured interview with the pupils regarding signs of respiratory tract infections, smoking habits, etc.

Definitions of exposure

Allergen exposure: Birch pollen exposure was defined as positive in children examined in May and June. Pet exposure was defined as positive in children who had a furred pet at home

Having a cold was defined as a positive answer to the question: 'Do you have a cold?' at the examination.

Building dampness was defined as a positive answer to the question: 'Does the bottom inside of double-glazed windows in the bedroom of your child usually contain more than 5 cm of condensed water during winter'.[20] Window pane condensation in dwellings is a dampness indicator, that is associated with both high relative indoor humidity (>45% RH), deficient ventilation (<0.5 air exchanges per hour), and increase of volatile organic compounds (VOC) in indoor air. Window condensation has also been associated with mould growth on the window frames,[21] and presence of house dust mite allergens in the dwelling.[22,23]

Passive smoking was defined as positive in children who reported having a smoking family member in the household.

Measurements of exhaled NO

The NO measurements were performed in accordance with the American Thoracic Society (ATS) recommendations for on-line NO measurements except for the target flow rate and the mouth wash procedure.[24] We used the Aerocrine NO system (Aerocrine AB, Stockholm, Sweden), including the CLD 77 AM chemiluminiscence analyser (Eco Physics AG, Dürnten, Switzerland) for on-line NO measurements and monitoring of flow and pressure. The sensitivity of the analyser is 0.1 ppb, the rise time 0–90% is <0.1 s. The sample flow rate 110 ml/s and the lag time from the mouthpiece 0.7 s. A three point calibration was performed before each study session using a certified calibration gas (10 ppm NO in N2; AGA AB, Älvsjö, Sweden). Before the experiments the mouth was rinsed during 20 s with 25 ml of 10% sodium bicarbonate.[25] Within 5 min of the mouth wash the children were comfortably seated, inhaled NO-free air from a reservoir and subsequently exhaled against a linear resistor (Hans Rudolph Inc. Kansas City, KS, USA). Mean oral pressure for all exhalations was 9.2 (± 0.4) cm H_2O. Three exhalations during 10 s each were performed consecutively at a target flow rate of 0.1 L/s with the help of visual guidance from the computer screen, and an average value was calculated. Values are presented as NO at the exhalation flow rate $= 0.1$ L/s ($FE_{NO0.1}$).

Skin prick tests

The skin prick testing was performed on the commonest air borne allergens in the area, *Dermatophagoides pteronyssinus*, cat, dog, birch pollen, timothy pollen. Sensitisation was defined as a positive prick test with a mean diameter of at least 3 mm. IgE sensitisation was defined as at least one positive prick test to the allergens above.

Ethics

The study was approved by the Ethical Committee of the Medical Faculty of Uppsala University.

Statistical methods

All data were fed into a Microsoft Office Access database and processed. Statistical analyses were performed using StatView 5.0 (SAS Institute Inc, Cary, NC, USA). Before analyses NO values were log-transformed and the results expressed as geometric mean with a 95% confidence interval. Unpaired *t*-test was used for comparisons between groups of the population. The associations between the exhaled level of NO and different explanatory variables were tested using multiple linear regression models. A P-value <0.05 was considered as statistically significant.

Results

Of the 374 children in this investigation 194 (51.9%) were girls, 92 (24.6%) had a cat, 73 (19.6%) had a dog and 61 (16.3%) had other furred pets at home. Almost half of the children (184 (49.3%) reported that they had a cold a the examination and 32 (8.8%) lived in houses with parental-reported window pane condensation. Five children reported that they smoked and 116 (31.1%) lived in homes where at least one family member was a smoker.

The mean intraindividual coefficient of variation (CV) of the measured $FE_{NO0.1}$ levels was 9.8% (9.0–10.6). The levels of exhaled NO was significantly increased in children sensitised to all individual inhalation allergens except mite (Table 1) and the exhaled NO levels increased with the number of allergens that the children were sensitised to (Fig. 1). Sensitisation to pets was a more important determinant of exhaled NO than sensitisation to pollen (Fig. 2).

There was a trend towards higher NO levels in children sensitised to birch or cat that were exposed to the sensitising allergen although this

Table 1 Prevalence of sensitisation and $FE_{NO0.1}$ levels (ppb, geometric mean (95% CI)) in relation to type of sensitisation.

	% sensitised	Not sensitised	Sensitised	*P*-value
Dog	12.3	5.4 (4.9–6.0)	19.4 (15.2–24.7)	<0.0001
Cat	18.4	5.2 (4.7–5.8)	14.9 (12.5–17.8)	<0.0001
Mite	3.2	6.3 (5.6–6.9)	9.5 (5.2–17.1)	0.15
Timothy	17.1	5.6 (5.0–6.2)	12.0 (9.4–15.3)	<0.0001
Birch	15.2	5.7 (5.2–6.4)	11.2 (8.8–14.3)	<0.0001
Any	33.7	4.6 (4.1–5.1)	11.9 (10.1–13.9)	<0.0001

difference was not statistically significant (Table 2). Cat-sensitised children that had a cat and/or other furred pets except dogs at home had significantly higher NO levels than cat-sensitised children with no furred animals at home ($P = 0.03$). No significant association was found between NO levels and dog exposure in dog-sensitised children (Table 2).

Non-sensitised children that reported having a cold or where the parents reported window pane condensation had higher levels of exhaled NO than non-sensitised children without a cold and not living in houses with window pane condensation, respectively (Figs. 3 and 4). These associations were not found in children that were sensitised to any inhalation allergen.

No significant association was found between exhaled NO levels passive smoking (6.1 (5.2–7.2 vs. 6.4 (5.7–7.2) ppb, $P = 0.70$) in exposed and non-exposed children, respectively).

When performing multivariable analyses the same pattern occurred as in the univariable analyses. In the non-sensitised children a significant relation was found between higher levels of exhaled NO and window pane condensation and having a cold, while in the allergic children a significant correlation between being sensitised to cat and/or dog and high exhaled NO levels was found (Table 3). All significant relations remained statistically significant when the analysis was limited to the children from the schools where all participants were included.

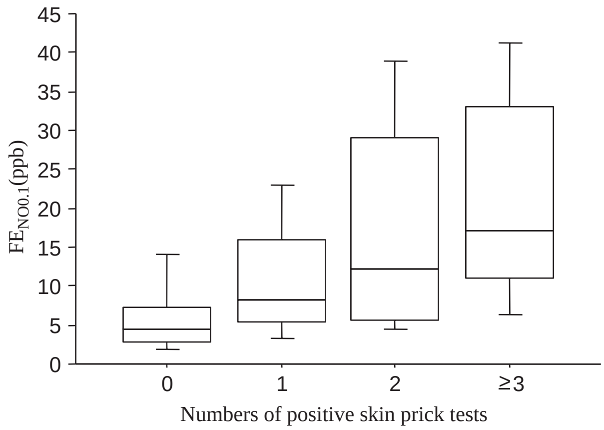

Figure 1 Levels of exhaled NO and FEV_1 in the relation to the number of positive skin prick tests (10th, 25th, 50th, 75th and 90th percentile).

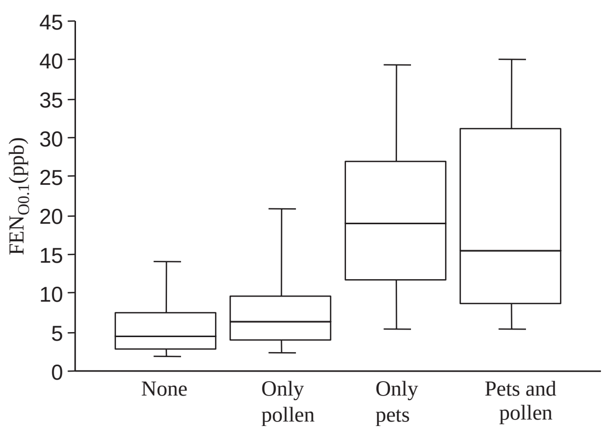

Figure 2 Levels of exhaled NO and FEV_1 in in relation to pollen and/or pet sensitisation (10th, 25th, 50th, 75th and 90th percentile).

Discussion

The main findings of this investigation are that among IgE-sensitised children exhaled NO levels are higher in children sensitised to perennial allergens (cat and dog) than in children that are only sensitised to seasonal allergens, whereas in non-sensitised children exhaled NO levels were significantly related to respiratory tract infections and window pane condensation.

It is well known that exhaled NO levels increase after allergen exposure[26] and as expected from previous studies, the NO levels were higher in IgE-sensitised than non-sensitised children.[27,28] In our study the highest levels of exhaled NO were found

Table 2 $FE_{NO0.1}$ levels (ppb, geometric mean (95% CI)) in relation to type of sensitisation and exposure to the sensitising allergen.

Sensitisation	Proportion exposed	Not exposed	Exposed	*P*-value
Dog sensitised	7/46	20.7 (9.0–48.1)	13.2 (7.3–23.8)	0.18
Cat sensitised	6/69	14.3 (11.9–17.1)	23.4 (10.2–53.7)	0.12
Cat sensitised and exposed to cat or other furred pets except dog	9/69	13.9 (11.5–16.7)	24.0 (14.5–39.7)	0.03
Birch sensitised	11/57	10.2 (15.8–27.2)	16.4 (8.9–30.1)	0.13

in children sensitised to pet allergens. We known from previous Swedish studies that cat and dog allergens are a constant finding in the school environment and can even be found in the homes of children where pets are not kept.[29,30] The increased levels of exhaled NO in pet-sensitised children are therefore probably related to allergen exposure. In the cat-sensitised children there was also a trend towards higher NO levels in children who had cats or other furred pets at home. In accordance with Henriksen and co-workers[11] we also found a trend towards higher exhaled NO levels in birch sensitised children that were investigated during the pollen season than those investigated during other months.

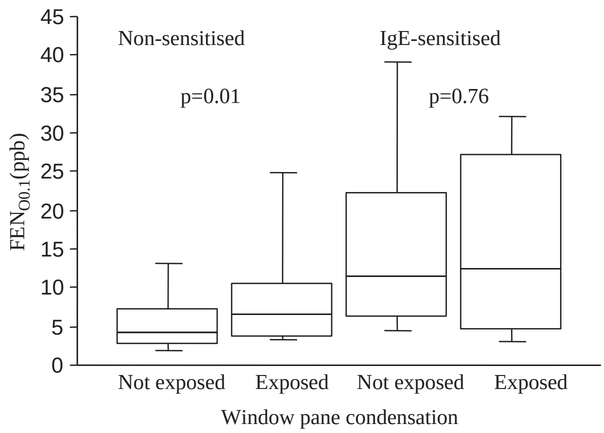

Figure 3 Levels of exhaled NO and FEV_1 in non-allergic and allergic children in relation to window pane condensation (10th, 25th, 50th, 75th and 90th percentile).

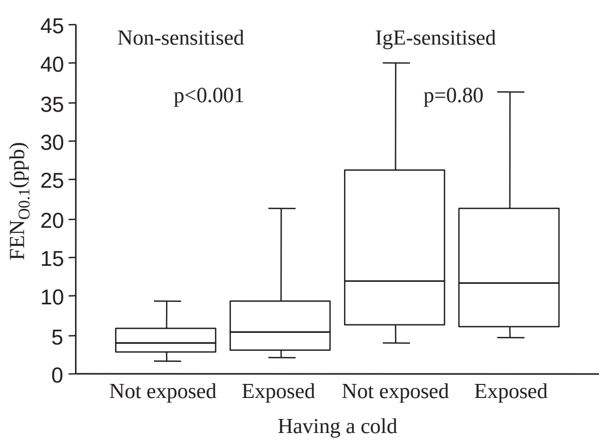

Figure 4 Levels of exhaled NO and FEV_1 in non-allergic and allergic children in relation to the having a cold at the examination (10th, 25th, 50th, 75th and 90th percentile).

Whereas allergen exposure seems to be the most important determinant for exhaled NO levels in allergic children, non-allergenic exposure seems to be more important in non-allergic children. In the non-allergic group, schoolchildren who reported having a cold had significantly higher $FE_{NO0.1}$ levels than children not having a cold. The fact that viral infections in the respiratory tract may influence the level of exhaled NO is known before,[14,15] but our results that this was found in non-sensitised children only is novel.

Our results showing a relationship between building dampness and airway inflammation, measured as exhaled NO, in non-sensitised children is potentially of great importance. There is a large amount of data showing an increased prevalence of respiratory symptoms in children and adults living in damp homes,[31,32] but the association between building dampness and exhaled NO has only been sparsely studied previously. In two studies from Finland no association was found between exhaled NO levels and working in mould-damaged buildings,[33,34] whereas Franklin et al. found an association between domestic formaldehyde levels and exhaled NO levels in a study of healthy children. In our investigation the association between window pane condensation and exhaled NO levels was only found in the group of non-allergic children. Window pane condensation is one indicator of building dampness, that has been validated by comparison with measured exposure. In previous studies, window condensation was associated with an increase of asthma in pre-school children.[19,35] In

Table 3 Independent effect on exhaled NO of different risk factors (the effect estimate is log-transformed and presented with a 95% confidence interval).

	Non-allergic ($n = 240$)	Allergic ($n = 121$)
Dog or cat		0.38 (0.24, 0.53)
Timothy		0.01 (−0.13, 0.15)
Birch		−0.03 (−0.18, 0.11)
Mite		0.06 (−0.19, 0,30)
Humidity	0.22 (0.06, 0.38)	−0.02 (−0.25, 0.28)
Cold	0.16 (0.07, 0.25)	−0.01 (−0.14, 0.13)

Adjusted for sex, type of home and the variables in the table.

dwellings, window pane condensation is an indicator of unhealthy indoor environment, with increased relative air humidity and an air exchange below the current ventilation standard of a minimum of 0.5 air exchanges per hour.[36] In addition, window condensation is associated with an increased risk for mite allergen and mould exposure.[20–22] This result is also in line with the results of a previous Swedish study that found a stronger association between building dampness and asthma in non-sensitised children than in children that were sensitised to inhalation allergens.[37]

In the present study we found no association between reported passive smoking and exhaled NO. These results are in contrast to those of Yates and co workers who found that exhaled NO decreases when healthy subjects are exposed to passive smoking in an experimental situation[38] and Warke et al. reported that lower levels of exhaled NO in asthmatic children with reported passive smoking exposure than in asthmatics with out passive smoking.[39] Warke and co-workers did, however, not find any association between passive smoking and exhaled levels of NO in healthy subjects[39] and like the present study the results were based on self-reported exposure which may cause problems with misclassification and quantification of the exposure.

We conclude that the determinants of exhaled NO levels are different in IgE-sensitised and non-sensitised children. Allergen exposure seems to be the most important determinant for exhaled NO levels in IgE-sensitised children whereas in non-sensitised children exhaled NO levels were associated to respiratory tract infections and window pane condensation, an indicator of building dampness and unhealthy indoor environment. As our exposure data was based on self- or parent-reported exposure, our results should be validated using objective exposure measurements.

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